Regulation of Rap1 signaling during Dictyostelium chemotaxis and development

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Chapter 5

Summary and Dissussion

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Summary and Discussion

Chemotaxis, cell motion directed by external chemical gradients, is a phenomenon of widespread occurrence and an essential property of various eukaryotic cells, including free-living microorganisms, leukocytes (inflammation), endothelial cells (angiogenesis), sperm (fertilization), neuronal growth cones (neurogenesis), fibroblasts (wound healing), thymocytes (embryogenesis), and *Dictyostelium discoideum* (chasing of food source and survival) (Ashcroft et al., 1999; Müller et al., 2001; Devreotes and Janetopoulos, 2003; Parent, 2004;). Therefore, further characterization of the molecular mechanism of chemotaxis is of great importance for understanding of many biological events. Mammalian neutrophils and the amoeba *Dictyostelium discoideum* are the most commonly used model systems to study eukaryotic chemotaxis (Van Haastert and Devreotes, 2004). In both mammalian neutrophils and *Dictyostelium*, chemotaxis is initiated by the binding of chemoattractants to cell surface G protein coupled receptors (GPCRs), leading to the release of Gα-GTP and Gβγ dimer (Figure 1). From a classical point of view, Gα subunits are considered to serve as "timer" to govern Gβγ signaling by releasing and re-associating Gβγ dimer from/to GPCRs, therefore less attention was drawn to Gα signaling. Recently, it has been realized that Gα plays an important role in transducing signal from GPCRs to downstream effectors. And more and more Gα-specific effectors in chemotaxis have been identified. In mammalian neutrophils, $G\alpha_i$ can interact with Elmo1/Dock180 (Li et al., 2013), mInsc (Kamakura et al., 2013), and Homer3 (Wu et al., 2015), while $G\alpha_{12/13}$ is able to bind to p115RhoGEF (Kozasa et al., 1998) and mTORC2 (Gan et al., 2012). In *Dictyostelium*, disruption of Gα2 results in cells that do not respond to cAMP stimulation and are unable to aggregate (Kumagai et al., 1991). Despite the essential function of Gα2 in cAMP-mediated chemotaxis, Gα2 had not been reported to directly activate downstream chemoattractant effectors in *Dictyostelium*. The activation of heterotrimeric G proteins induces a rapid and defined cell polarity, in which F-actin is polymerized at the leading edge of the migrating cell, leading to pseudopod extension at the membrane surface which drives cells moving forward, whereas myosin II is concentrated at the cell's posterior, functioning as contraction force causing cells to de-adhere (Ridley et al., 2003; Swaney et al., 2010; Artemenko et al., 2014). This highly organized and coordinated process requires the participation of small G proteins. Small G proteins switch between inactive GDP-bound and active GTP-bound states. Only in the GTP-bound state can small G-proteins interact with downstream effectors. This GDP–GTP cycle is highly regulated by two categories of proteins: guanine nucleotide-exchange factors (GEFs) and GTPase-activating
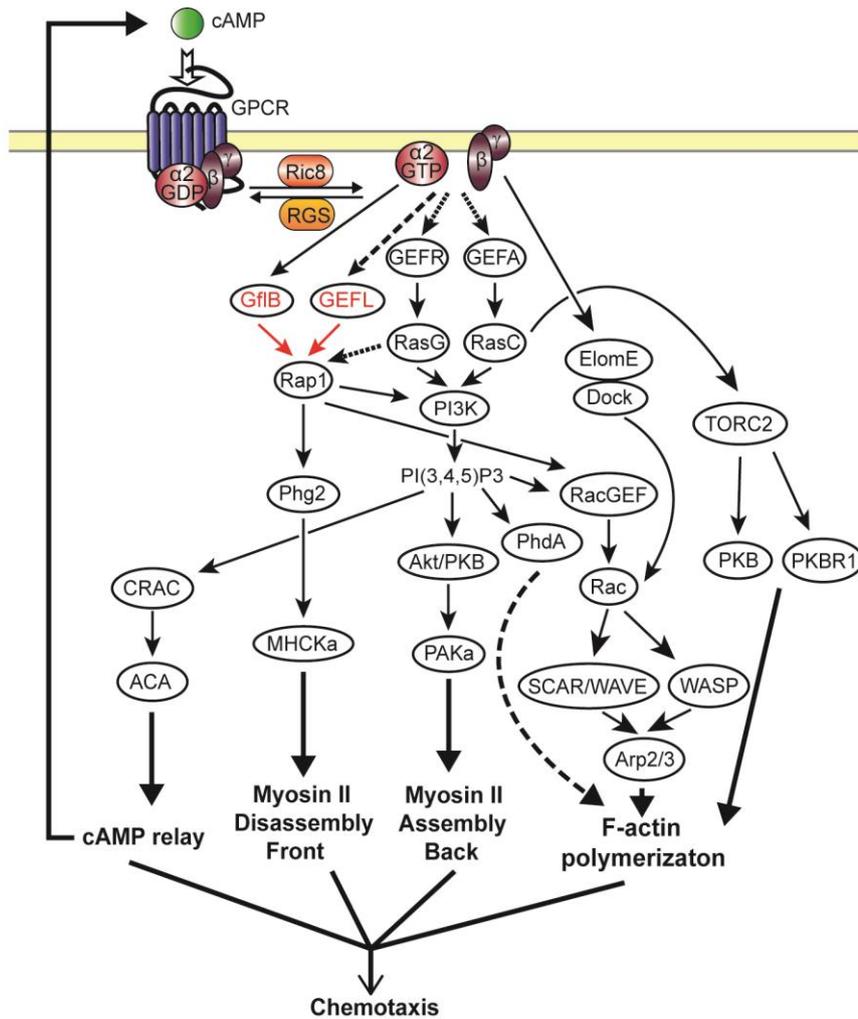
Figure 1. A schematic representation of the chemotactic signaling pathways in *Dictyostelium discoideum*. Upon stimulation of cAMP, heterotrimeric G proteins undergo conformational changes to disassociate into Gα2-GTP and a Gβγ dimer, both of which are able to interact with various downstream effectors. Thus far, GflB and ElomE are the only two identified proteins in *Dictyostelium* that directly interact with Gα2-GTP and Gβγ, respectively.

proteins (GAPs) (Bos et al., 2001). GEFs facilitate release of the bound nucleotide and allow the more abundant GTP to rebind, whereas GAPs stimulate its low intrinsic GTPase activity to stimulate the hydrolysis of the bound GTP to complete the cycle. In *Dictyostelium*, the small G-proteins Ras and Rap1 are rapidly and transiently activated at the leading edge in response to chemoattractant stimulation (Sasaki et al., 2004; Jeon et al., 2007a, 2007b; Kortholt and van Haastert, 2008; Kortholt et al., 2013). However, how heterotrimeric G-proteins induce symmetry breaking in Ras and Rap1 signaling was not well understood. In

this thesis, we described the identification and characterization of a novel Rap specific GEF, GflB, which is specifically binding to and activated by *Dictyostelium* Gα2 (Chapter 2). Additionally, two newly found Rap1-specific GEFs were reported in Chapter 3.

Rap1 is regulated by multiple GEFs in chemotaxis

In *Dictyostelium*, Rap1 is rapidly and transiently activated at the leading edge in response to chemoattractant stimulation in a pattern that is much broader than Ras-GTP (Jeon et al., 2007a). In cells lacking the two major chemotaxis Ras proteins ($rasC^-$/$rasG)^-$ this cAMP-mediated activation is completely abolished, suggesting that Rap1 activation occurs downstream of Ras (Bolourani et al., 2008). However, no direct interaction between Ras and regulators of Rap1 activation have been established so far. At the start of my project only *Dictyostelium* GbpD had been identified as Rap1 specific GEF (Kortholt et al., 2006). During this thesis I identified three additional GEFs for Rap1, GflB (Chapter 2), GefQ and GefL (Chapter 3) that together with GbpD regulate Rap1 activation during the *Dictyostelium* life cycle (Figure 2). Taken together, we propose that GbpD and GefQ regulate Rap1 activity in vegetative growth, whereas GflB and GefL are the major regulators of Rap1 during multicellular development and cAMP-induced chemotaxis. In addition, GefQ appears to
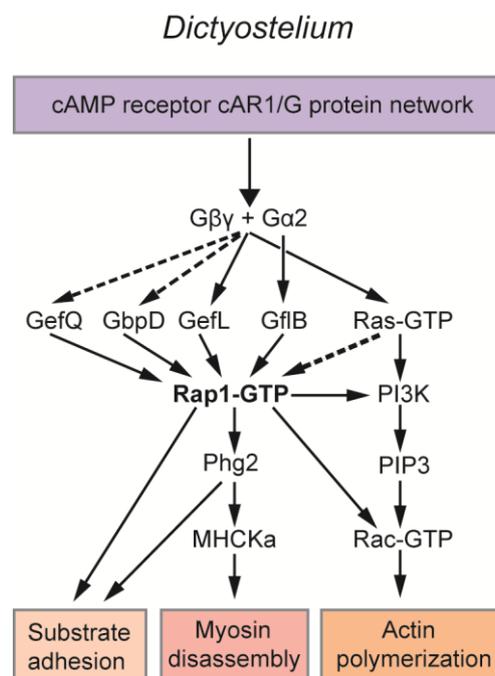
Figure 2. A schematic representation of Rap1 signaling pathways in *Dictyostelium*. *Dicytostelium* Rap1 is at least activated by four GEFs during various development states. Rap1 functions in substrate adhesion and cytoskeleton rearrangement via distinct downstream effectors.

mainly contribute to Rap1 activation during *Dictyostelium* cytokinesis (Plak et al., 2014). GflB was identified in a proteomic screen in *Dictyostelium* by using purified Gα2 protein as a bait (Kataria et al., 2013). GflB directly binds to Gα2 preferentially in the GTP-bound state (Chapter 2). Further investigation revealed that GflB is a Gα2-stimulated Rap1 specific GEF that is required for efficient directional sensing and cell movement during chemotaxis. GflB plays an important role in regulating the balance between Ras- and Rap1-mediated F-actin and myosin dynamics during chemotaxis. Therefore, GflB forms a direct connection between heterotrimeric G protein and monomeric G protein signaling.

RasGefL is also an important regulator of cAMP-mediated Rap activation (Plak et al., 2016, MS submitted, chapter 3 in this thesis). Disruption of *gefL* resulted in severely impaired Rap1 activation and decreased chemotaxis and motility towards the cAMP source. Additionally, GefL plays a role in phototaxis, thermotaxis, and spores viability during the late developmental stages (Wilkins et al., 2005, chapter 3). Cells lacking *gefL* have a severely impaired cAMP-mediated Rap response, but normal kinetics of Ras activation. Considering the dependency of Rap1activition on RasC/RasG (Bolourani et al., 2008) and normal kinetics of Ras activation in *gefL*-null cells this suggests that GefL is regulated downstream of Ras proteins. However, full length GefL protein does not directly interact with Ras and Rap1 proteins, suggesting RasC or RasG indirectly activates GefL.

Cells lacking *gbpD* have a hyperpolar and elongated morphology, that exhibit enhanced migration speed accompanied by decreased substrate adhesion (Bosgraaf et al., 2005). Overexpression of GbpD complements the *gbpD⁻* mutant chemotactic defect, but also induces cells to extend many substrate-attached pseudopodia (Bosgraaf et al., 2005). This phenotype is similar to that of cells overexpressing Rap1 (Rebstein et al., 1993). As discussed in chapter2, our work suggests the existence of a positive feedback loop of PIP3/GbpD/Rap1/PI3K/PIP3 in GbpD regulation, of which PIP3 serves as a both upstream activator and downstream effector of GbpD/Rap1signaling. This thus potentially explains the mentioned strong phenotypes of GbpD overexpression.

RasGefQ was previously reported as a GEF protein for *Dictyostelium* RasB *in vivo* (Mondal et al., 2008). However, by performing *in vitro* nucleotide exchange assays using highly stable catalytic region protein from GefQ it was shown that GefQ stimulates the exchange on Rap1, but not on RasB, RasC, RasD, and RasG (Chapter 3), indicating that GefQ specifically activates Rap1 *in vitro*. Comparison of the previous report (Mondal et al., 2008)

and our studies reveal that vegetative *gefQ*-null cells have severally decreased Rap1 activation (Plak et al., 2016, MS submitted) and normal level of RasB activation (Mondal et al., 2008), whereas starved *gefQ*-null cells show impaired RasB activity (Mondal et al., 2008) and normal level of active Rap1 (Plak et al., 2016, MS submitted). We therefore propose a model in which GefQ mainly stimulate Rap1 in vegetative cells, while in starved cells RasB may be the main target of GefQ. During vegetative growth, GefQ mediated Rap1 signaling is important for regulating cytoskeletal arrangement during cell division and folic acid induced cell movement (Chapter 3). In starved cells, GefQ specifically activates RasB, which plays a role in developmental patterning and slug mobility (Mondal et al., 2008).

Conclusion and outlook

Taken together our results and previous data show that *Dicytostelium* Rap1 is activated by at least four GEFs during the different development states. These GEFs together are key for the balance in the temporal and spatial activation of Ras and Rap1. Although the regulation of these GEFs is still not completely understood, especially for GefL, they all play a role in regulating Rap1 in response to both intracellular and extracellular stimuli, which is crucial for various cellular processes, including adhesion, cytokinesis, and chemotaxis. Thus far, the direct mediators between Ras activation and Rap1 activation have not been established. However our work has shown that GefL is an essential link in this connection. Therefore, a mass spectrometry based proteomic approach with GefL as bait might be beneficial in these enterprises (Kataria et al., 2013).

Importantly our data also revealed a direct connection between heterotrimeric G protein and *Dicytostelium* Rap1. GflB (Liu et al., 2016) binds like the mammalian ELMO1/Dock180 complex specifically to Gα-GTP. In breast cancer cells $Gα_{i2}$-GTP specifically recruits the ELMO1/Dock180 complex to the cell membrane leading thereby inducing Rac1/2 activation and subsequently actin polymerization and cancer cell migration (Li et al., 2013). Together these observations suggests that GTP bound Gα2 is the active form of the protein. However in contrast, LGN/AGS3 does not interact with active Gα-GTP, but specifically binds $Gα_{i2}$-GDP at the leading edge of migrating neutrophils (Kamakura et al., 2013). Therefore, it's still unclear if Gα2-GDP, Gα2-GTP or both free Gα- GDP and GTP represent the active form. To further investigate this it will be instrumental to have better

cellular markers to monitor the nucleotide bound state *in vivo* and identify and characterize additional regulators and effectors of Gα.

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